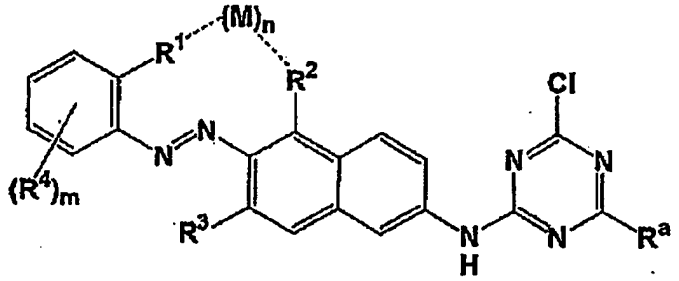
AMENDMENTS TO THE CLAIMS

This listing of the claims will replace all prior versions, and listings, of claims in the

Listing of Claims:

1. (Currently amended) A method of visualizing a protein bound to a protein-binding membrane, said method comprising:

(1) staining at least one protein bound to a protein-binding membrane with an effective amount of a staining reagent comprising at least one compound of formula I:



I

wherein,

R^a is selected from halogen and -NH-Ar;

R^1 and R^3 are independently selected from the group consisting of -OH, -COOH and $-SO_3H$;

R^2 is selected from the group consisting of -OH and -SH;

R^4 is selected from the group consisting of -COOH, $-SO_3H$, $-NH_2$, $-NH(C_1-C_6)$alkyl, -NHacyl, -NHAr, -OH and -O-acyl;

m is 0 or 1;

M is a transition metal selected from the group consisting of chromium, manganese, iron, cobalt, nickel, copper, zinc, cadmium and combinations thereof;

n is 0 or 1;

- - - - - - indicates coordination to the transition metal M; and

Ar is unsubstituted phenyl or substituted phenyl;

wherein the substituents for Ar are selected from the group consisting halogen, $-NO_2$, and $-SO_3H$;

or a salt of such a compound;

(2) incubating said protein bound to the protein-binding membrane with said staining reagent for a time interval sufficient to allow reaction of the protein with the staining reagent to yield a stained protein;

(3) destaining said staining reagent from the protein-binding membrane; and

(4) observing the stained protein,

wherein about 2 ng of the stained protein are visually observable.

2. (Original) The method of claim 1 wherein:

R^a is halogen;

R^1 is -COOH;

R^2 is -OH;

R^3 is $-SO_3H$;

m is 0;

M is chromium; and

n is 0 or 1;

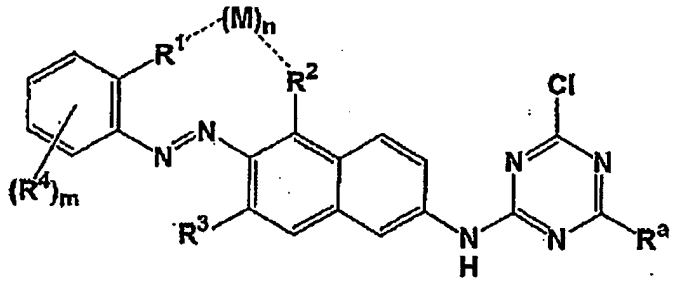
or a salt of such a compound.

3. (Original) The method of claim 2 wherein the staining reagent comprises reactive brown 10, or a salt thereof.

4. (Original) The method of claim 1, wherein the protein-binding membrane is selected from the group consisting of nitrocellulose, nylon and polyvinylidene difluoride.

5. (Original) The method of claim 1 wherein the process is performed at room temperature.

6. (Currently amended) A composition comprising at least one protein bound to a protein-binding membrane, which protein has been stained with a staining reagent comprising at least one compound of formula I:



I

wherein,

R^a is selected from halogen and -NH-Ar;

R^1 and R^3 are independently selected from the group consisting of –OH, -COOH and $-SO_3H$;

R^2 is selected from the group consisting of -OH and –SH;

R^4 is selected from the group consisting of -COOH, $-SO_3H$, $-NH_2$, $-NH(C_1-C_6)$alkyl, -NHacyl, -NHAr, -OH and –O-acyl;

m is 0 or 1;

M is a transition metal selected from the group consisting of chromium, manganese, iron, cobalt, nickel, copper, zinc, cadmium and combinations thereof,

n is 0 or 1;

- - - - - - indicates coordination to the transition metal M; and

Ar is unsubstituted phenyl or substituted phenyl;

wherein the substituents for Ar are selected from the group consisting halogen, $-NO_2$, and $-SO_3H$;

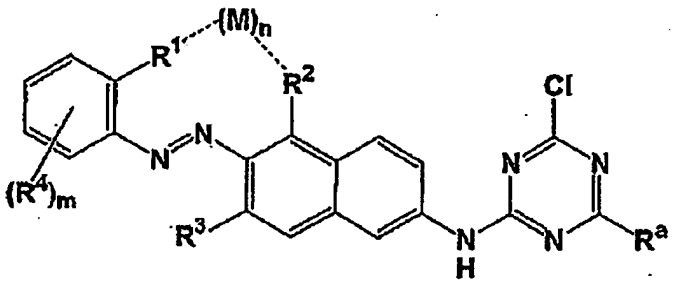
or a salt of such a compound,

wherein ~~the staining reagent comprising at least one compound of formula I is not covalently bound to the protein-binding membrane~~ said protein-binding membrane is capable of being destained, and wherein about 2 ng of said protein are visually observable when the protein-binding membrane is destained.

7. (Original) The composition of claim 6 wherein the staining reagent comprises reactive brown 10.

8. (Original) A method of reversing the staining procedure of claim 1, comprising:

(1) providing a protein-binding membrane having at least one protein spot bound thereto stained with a staining reagent comprising at least one compound of formula I:



I

wherein,

R^a is selected from halogen and -NH-Ar;

R^1 and R^3 are independently selected from the group consisting of -OH, -COOH and $-SO_3H$;

R^2 is selected from the group consisting of -OH and -SH;

R^4 is selected from the group consisting of -COOH, $-SO_3H$, $-NH_2$, $-NH(C_1-C_6)$alkyl, -NHacyl, -NHAr, -OH and -O-acyl;

m is 0 or 1

M is a transition metal selected from the group consisting of chromium, manganese, iron, cobalt, nickel, copper, zinc, cadmium and combinations thereof;

n is 0 or 1;

- - - - - - indicates coordination to the transition metal M; and

Ar is unsubstituted phenyl or substituted phenyl;

wherein the substituents for Ar are selected from the group consisting halogen, $-NO_2$, and $-SO_3H$;

or a salt of such a compound;

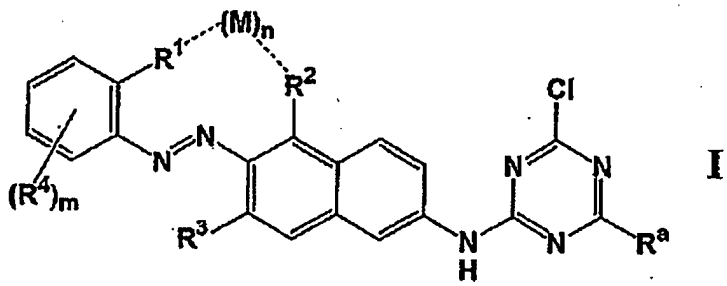
(2) incubating said protein-binding membrane with an aqueous alkaline solution; and

(3) washing the protein-binding membrane to remove the staining reagent.

9. (Original) The method of claim 8 wherein the staining reagent is reactive brown 10.

10. (Currently amended) A method of quantifying a protein analyte, comprising:

(1) staining at least one protein analyte spot and a series of protein standard spots of known quantity bound to a protein-binding membrane with a staining reagent comprising at least one compound of formula I:



I

wherein,

R^a is selected from halogen and -NH-Ar;

R^1 and R^3 are independently selected from the group consisting of -OH, -COOH and $-SO_3H$;

R^2 is selected from the group consisting of -OH and -SH;

R^4 is selected from the group consisting of -COOH, $-SO_3H$, $-NH_2$, $-NH(C_1-C_6)$alkyl, -NHacyl, -NHAr, -OH and -O-acyl;

m is 0 or 1;

M is a transition metal selected from the group consisting of chromium, manganese, iron, cobalt, nickel, copper, zinc, cadmium and combinations thereof;

n is 0 or 1;

- - - - - - indicates coordination to the transition metal M; and

Ar is unsubstituted phenyl or substituted phenyl;

wherein the substituents for Ar are selected from the group consisting halogen, $-NO_2$, and $-SO_3H$;

or a salt of such a compound;

(2) incubating said protein analyte spot and said protein standard spots bound to the protein-binding membrane with the staining reagent for a time interval sufficient to allow reaction of the protein spot and said protein standard spots with the staining reagent;

(3) destaining the staining reagent from the protein-binding membrane, wherein about 2 ng of said protein are visually observable;

(4) generating image quantification data for the known protein standard spots and for the protein analyte spot;

(5) constructing a standard calibration curve using the known concentrations of the protein standard and the corresponding image quantification data; and

(6) calculating a concentration for the protein analyte.

11. (Original) The method of claim 10, wherein the protein-binding membrane is selected from the group consisting of nitrocellulose, nylon and polyvinylidene difluoride.

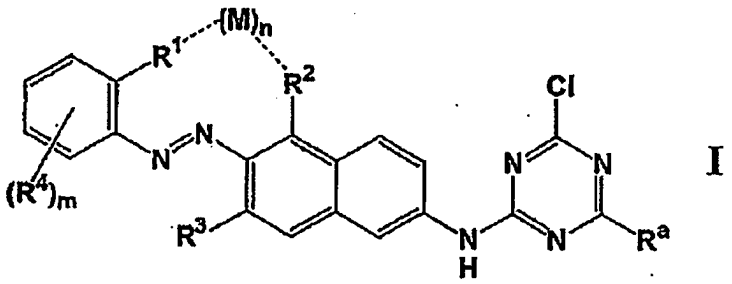
12. (Original) The method of claim 10 wherein the protein standard is bovine serum albumin.

13. (Original) The method of claim 10 wherein the staining reagent comprises reactive brown 10.

14. (Currently amended) A kit for visualizing a protein bound to a protein-binding membrane comprising:

(1) one or more protein-binding membranes; and

(2) a staining reagent comprising at least one compound of formula I:



I

wherein,

R^a is selected from halogen and -NH-Ar;

R^1 and R^3 are independently selected from the group consisting of –OH, -COOH and $-SO_3H$;

R^2 is selected from the group consisting of -OH and –SH;

R^4 is selected from the group consisting of -COOH, $-SO_3H$, $-NH_2$, $-NH(C_1-C_6)$alkyl, -NHacyl, -NHAr, -OH and –O-acyl;

m is 0 or 1;

M is a transition metal selected from the group consisting of chromium, manganese, iron, cobalt, nickel, copper, zinc, cadmium and combinations thereof;

n is 0 or 1;

- - - - - - indicates coordination to the transition metal M; and

Ar is unsubstituted phenyl or substituted phenyl;

wherein the substituents for Ar are selected from the group consisting halogen, $-NO_2$, and $-SO_3H$;

or a salt of such a compound,

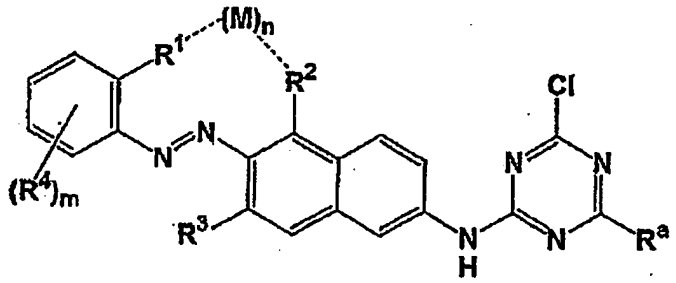
wherein ~~the staining reagent comprising at least one compound of formula I is not covalently bound to the protein-binding membrane~~ said one or more protein binding membranes are capable of being destained, and wherein said staining reagent is capable of making about 2 ng of a protein visually observable.

15. (Original) A kit according to claim 14 wherein the staining reagent comprises reactive brown 10.

16. (Currently amended) A kit for quantifying an amount of a protein, comprising:

(1) one or more protein-binding membranes;

(2) a staining reagent comprising a at least one compound of formula I:



I

wherein,

R^a is selected from halogen and -NH-Ar;

R^1 and R^3 are independently selected from the group consisting of -OH, -COOH and $-SO_3H$;

R^2 is selected from the group consisting of -OH and -SH;

R^4 is selected from the group consisting of -COOH, $-SO_3H$, $-NH_2$, $-NH(C_1-C_6)$alkyl, -NHacyl, -NHAr, -OH and -O-acyl;

m is 0 or 1

M is a transition metal selected from the group consisting of chromium, manganese, iron, cobalt, nickel, copper, zinc, cadmium and combinations thereof;

n is 0 or 1;

- - - - - - indicates coordination to the transition metal M; and

Ar is unsubstituted phenyl or substituted phenyl;

wherein the substituents for Ar are selected from the group consisting halogen, $-NO_2$, and $-SO_3H$;

or a salt of such a compound; and

(3) a set of one or more solutions of a protein standard of known concentration,

wherein the staining reagent may be used to detect about 2 ng of a protein.

17. (Original) A kit according to claim 16 wherein the staining reagent comprises reactive brown 10.